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A CONTRIBUTION TO THE LIFE HISTORY OF *CASUARINA*

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Introduction

The genus *Casuarina* Linn. consists of about 35 species distributed chiefly in Australia and Polynesia (Willis, 1931). From its center of origin it has migrated to regions of greater humidity and heat, tropical or equatorial, such as New Caledonia and the islands of the Asiatic Archipelago. Of all the species of this genus only *C. equisetifolia* Forst. actually possesses an extended distribution, which comprises all the lands about the Indian Ocean. In India, it occurs in a wild state on the eastern side of the Bay of Bengal (Hooker, 1894). Owing to its fast-growing nature, its capability for growing on coastal areas, and the importance it has in the reclamation of sand dunes, it has become one of the prominent cultivated trees elsewhere, especially on the Coramandel and Kanara Coasts (Gamble, 1922). In the interior, it is grown mainly for fuel; recently, the tall trunks have begun to be used as poles and rafters and hence have attained an economic status. Two other species, *C, suberosa* Ott. & Dietr. and *C. stricta* Ait, which have been introduced into India, are less successful and are restricted to small areas in the Nilgiris, where they are grown in plantations at an elevation between 5000 and 7000 feet.

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There is some difference of opinion regarding sex expression in *C. equisetifolia.* Hooker (1894) has called it "dioecious (?)"; but according to Brandis (1906), it is monoecious and "male and female flowers are sometimes found on the same branch, but, (as often happens with monoecious trees) some trees habitually bear male flowers, others female only." Rendle (1938) also says that the plant is monoecious. In plantations near Bangalore (India), however, dioecism is the prevailing type of sex expression and monoecism is rare.

The staminate flowers are borne in terminal spikes on short lateral branches. The internodes of the inflorescence axis are very short and at each node the bracts fuse to form a cup, with several flowers hanging out over its edge. Each of these flowers has two bracteoles representing the outer perianth and two membranous and transparent flap-like structures, placed at right angles to the former, representing the inner perianth. The filament originates from the base of the inner whorl and bears at its tip a single anther with four loculi (figs. 1–4). The female flowers are borne in dense globular or oblong heads. Each flower lies in the axil of a bract and has two bracteoles (figs. 5–7). The ovary is flattened laterally and consists of two fused carpels, with the posterior loculus empty and the anterior alone containing two ovules. The long and bifid stigma hangs out beyond the other floral parts.

In monoecious plants, the male flowers are borne towards the apex of lateral branches and the female cones in the axils of the latter.

Pollination is accomplished by the agency of wind during the months of September and October. As the fruit ripens, the two bracteoles, which are somewhat hairy during the early stages, develop into two large woody valves. The fruit is a one-seeded winged nut, the embryo completely filling the seed cavity. The seeds are disseminated during the months of January and February of the succeeding year.

Previous Work on the Genus

The first and the most important contribution on the embryology of *Casuarina* was published by Treub in 1891. The species investigated by him in greatest detail was *C. suberosa;* but a few stages in the development of two other species, *C. Rumphiana* and *C. glauca,* were also studied. He reported several unique features, the most significant of them being the origin of the basal part of the sporogenous tissue from cells not belonging to the archesporium but to the chalaza; the transformation of some of the cells of the sporogenous tissue into tracheids, a feature recalling the formation of elaters in certain liverworts; the simultaneous development of a score of embryo sacs to

maturity; the absence of antipodals; the formation of as many as fifty endosperm nuclei before fertilization; and the remarkable phenomenon of chalazogamy.

Some of Treub's observations were, however, so unexpected that in reviewing them Chamberlain (1896) suggested a study of the genus "in much greater detail, in order that Treub's conclusions may receive additional confirmation or be rejected."

Juel (1903) studied the development of the megaspores in *C. quadrivalvis* and an undetermined species collected from Algeria. In both species he demonstrated the formation of normal megaspore tetrads and also gave a rough estimate of the number of chromosomes saying that it was not less than eight and not more than twelve in the gametophytic cells. In addition, he drew attention to certain kinoplasmic bodies lying near the poles of the spindles during tetrad formation, which resemble the dense areas described in various gymnosperms. Though he did not pursue his observations to the later stages, he regarded the development of the embryo sac, the course of the pollen tube, and the stages in the formation of the embryo as conforming with the description of Treub.

Frye in 1903 gave an account of the development of the embryo sac (up to the time of fertilization) in *C. stricta*. He found that the development of the female gametophyte is quite normal and that the polar nuclei never form any endosperm before fertilization. He confirmed the presence of chalazogamy, but the course of the pollen tube within the nucellus and the exact place where its contents are discharged into the embryo sac were not studied by him.

As no work has been done during the last 45 years and as many of the features in its embryology are still obscure, it appeared to me that a fresh study was desirable.

Material and Methods

Material for the present investigation was collected from the *Casuarina* plantations near Bangalore. Formalin-acetic-alcohol and Allen's modification of Bouin's fluid were used for fixation. The usual method was followed for dehydration and infiltration and sections were cut serially with a sliding microtome. Heidenhain's iron-alum haematoxylin with a counterstain of light green or eosin in clove oil was used for staining.

To follow the course of the pollen tube, the following method was found useful: fresh carpels were dissected out and boiled in water for two minutes. They were then run up to pure glycerine through four stages, at an interval of two hours between each stage, in a thermostat

maintained at 52° C. The carpels were then mounted in lactophenol, which was slightly colored with aniline blue or safranin. The wall of the pollen tube absorbed these stains well and stood in sharp contrast with the rest of the tissue, making it possible to follow its course into the nucellus and embryo sac.

Observations

Casuarina equisetifolia

(1) *Microsporangium and male gametophyte.*—A transverse section of a young anther shows that the four groups of archesporial initials differentiate at an early stage, one at each corner. Each group is hypodermal in origin and consists of two or three cells as seen in transverse sections. The number of such cells as counted in longitudinal section varies between ten and fifteen per loculus.

The archesporial cells divide periclinally to form two layers (fig. 12); subsequent divisions of the outer or primary parietal layer result in about three or four layers of cells (fig. 13, 14). Of these, the outermost layer transforms itself into the endothecium and the innermost into the tapetum. The former develops the characteristic banded thickenings at maturity (fig. 16). The tapetal cells are sometimes binucleate (fig. 15).

The nuclei of the microspore mother cells undergo the usual prophasic changes of the two meiotic divisions to form tetrahedral tetrads. After a period of rest and the laying down of the exine and intine, the microspore (fig. 17) divides (fig. 18) to form two unequal cells, the larger vegetative and the smaller generative (fig. 19). Although spindle-shaped at first, the generative cell later assumes a spherical form and is seen to lie inside the cytoplasm of the vegetative cell (fig. 20). The mature pollen grain has a thick exine with three germ pores distributed equidistantly on the equator. This is the shedding stage of the pollen.

(2) *Ovary and ovule.*—The earlier development of the ovary reveals that it arises as a cup-shaped invagination of the thalamus. The edges of the cup converge, thus enclosing a cavity within. Above this point, the edge of the cup bifurcates and the two halves extend out of the bract in the form of solid filiform appendages, which are the stigmas (fig. 5, 6). After pollination is accomplished, the portion of the ovary enclosed within the bract expands and flattens and the stigmas dry up (fig. 8). The other histological changes which follow pollination will be considered later.

A very young ovary is represented in figure 9. It has two carpels

of which only one is fertile and the other aborts (see figs. 10, 11). Two epitropous[1] ovules originate in the former from the placenta. The inner integument, which is thinner (2–3 layers thick in the region of the micropyle, fig. 23), arises first but eventually becomes crushed between the outer integument and the nucellus, except in the region which forms the micropyle.

(3) *Megasporangium.*—At the time of origin of the two integuments, a plate of 5–7 hypodermal cells becomes differentiated as the archesporium. A longitudinal section of the nucellus at about this stage is represented in figure 21, which shows three such cells. Each of these cells divides periclinally to form a primary parietal and a primary sporogenous layer.

The primary parietal layer divides periclinally as well as anticlinally to form 5–6 layers of wall cells, as a consequence of which the sporogenous mass is pushed deeper into the nucellus. During this period, the primary sporogenous cells also undergo repeated divisions so as to constitute a compact mass of 40–50 cells occupying the axial region of the nucellus (fig. 23).

Meanwhile a procambial strand becomes differentiated in the chalaza. To begin with, this strand is merely an extension of the provascular trace supplying the placenta; later it extends upward into the center of the mass of sporogenous cells (fig. 22, 23). It may be noted that certain centrally situated cells of the sporogenous mass also seem to contribute towards the extension of the strand. In the early stages, the cells of the strand are distinguishable only by their elongated appearance, but by the time of fertilization such of the cells of the strand that lie outside the nucellus develop into vascular elements (figs. 32, 38). The nucellar cells which lie in continuation with the strand, however, become disorganized at the time when the embryo sacs send out antipodal caeca.

After the divisions of the primary sporogenous tissue, the resultant cells elongate and function as megaspore mother cells. Each megaspore mother cell shows the typical prophasic stages of meiosis and forms dyad cells (fig. 24), which in turn complete the second meiotic division to form a linear tetrad of megaspores (fig. 25).

Quite normally more than one megaspore of a tetrad begins further development. There is no definite rule as to which megaspore or megaspores should continue development; one, two, three or occasionally all the four may develop further. Some may develop only up to the two- or four-nucleate stages but others proceed right up to the eight-nucleate stage although the nuclei may not always become

[1] See B. D. Jackson's "A Glossary of Botanic Terms," 1900, London, p. 92.

organized into the various components of the embryo sac. In figure 27 all the megaspores of two adjacent tetrads have developed to the eight-nucleate stage. As many as 15–20 well organized embryo sacs are frequently present in the same nucellus (fig. 32).

At the two-nucleate stage the embryo sac usually begins to elongate towards the chalaza in the form of a long narrow tube (figs. 26–29). This tube or caecum forms a large terminal pouch at its lower extremity (figs. 31, 32). The initiation of the caecum may sometimes be postponed to the four- or even the eight-nucleate stage (fig. 30).

It may be recalled here that the ovary contains two ovules. Both of these develop together until the megaspore tetrad stage, The further development of an ovule is related to the presence of the pollen tube. The ovule which does not receive a pollen tube becomes arrested and is eventually crushed by the growth of its fellow.

The egg apparatus exhibits a variety of forms. The synergids may be hooked and have the usual basal vacuoles (figs. 33, 34), or they may look very much like the egg (fig. 35). In the latter case, they show an apical vacuole with the cytoplasm and nucleus lying towards the base. All these variations are often present in the embryo sacs belonging to the same nucellus. The polar nuclei are usually found attached to each other just below the egg apparatus, although sometimes they may occupy a different position in the embryo sac. The antipodals do not form cells and lie in the basal end of the caecum (figs. 31, 32).

(4) *Pollen tube.*—Pollination takes place during the months of September and October. The bifid and filiform stigmas secrete a mucilaginous substance which catches the pollen grains (fig. 36). The latter begin germination on the stigma within three to four days after pollination. The growth of the pollen tube is completely intercellular. It travels through the stigma, partly digesting the adjacent cells which are richly packed with nutritive materials. Occasionally the tube sends out short, blind branches towards the free end of the stigma (fig. 36).

As the tube leaves the stigma and enters the style, the former begins to dry up and wither away. The further course of the pollen tube is shown in figure 37, which is a sagittal section of the pistil of *C. equisetifolia.*

In the style, the pollen tube travels through the central region, where the cells are greatly elongated. This region is continuous with the ovule through the "bridge"[2] (see figs. 10, 11, 37, 38). It then

[2] For the sake of description, this term is used in the present paper to denote the tissue connecting the upper portion of the ovule with the ovary, so that the apex of the ovule is continuous with the style.

enters the chalazal region of the ovule. Here, another unique feature is presented. After arriving at this point, the pollen tube sends out several branches which end blindly. The manner of branching and the number of branches given out are not constant. The pollen tube represented in figure 38 (reconstructed from six serial sections) may be considered as typical. Some of the branches reach the outermost layers of cells of the outer integument towards the base; and while doing so, find their way between the chalazal pouches of the embryo sac caeca.

The particular branch of the pollen tube which contains the sperms makes its way between the caeca into the nucellus. Here the lumen of the tube becomes slightly expanded and its wall appears to be thicker. It absorbs such stains as eosin, erythrosin, and fast green with great avidity and therefore stands out sharply from the other tissues. Making its way upward between the embryo sacs, it reaches the upper end of one of them lying nearest to the micropyle (fig. 38).

(5) *Fertilization.*—The generative cell divides in the pollen tube before the latter enters the chalaza. The two sperm nuclei are each surrounded by a globular cytoplasmic sheath but no cell wall or enclosing membrane could be made out. The vegetative nucleus and the two sperms are always seen together, the former being sometimes slightly ahead of the sperms. Figure 40 represents the tube nucleus and the two male cells in the tip of a pollen tube which was on its way upward to the micropylar end of an embryo sac inside the nucellus.

The pollen tube enters the embryo sac at a point close to the egg apparatus and discharges the sperms. At this time the cytoplasmic sheath around them is not distinguishable.

One of the sperm nuclei fuses with the egg nucleus which is nearly two or three times larger than the former. It appears, however, that after a short time the second sperm nucleus undergoes some increase in size (fig. 39; note the difference in size between the sperm nucleus in contact with the polar nuclei and that inside the egg).

(6) *Endosperm.*—The fertilized embryo sac enlarges greatly in size while the unfertilized ones degenerate and disappear. This is clearly seen in figure 41, in which there is a fertilized sac with the endosperm nuclei in division and at its base, a small unfertilized sac on its way to disappearance.

The primary endosperm nucleus begins to divide soon after triple fusion is complete. All the early divisions take place in the micropylar part of the embryo sac. The daughter nuclei then move towards the periphery of the sac and continue further divisions, which gradually extend towards the chalazal region (fig. 42).

Wall formation commences at the micropylar end of the sac and proceeds centripetally as well as towards the chalaza. It is very common to find endosperm cells at the micropylar end and actively dividing nuclei at the chalazal end of the same embryo sac (fig. 43).

Broadly speaking, the endosperm may therefore be said to develop in four stages:

(a) During the early divisions, the spindles are evanescent and a large number of free nuclei are formed (fig. 42).

(b) In the second phase, free nuclear divisions cease in the micropylar region and wall formation commences from this end (fig. 43). The nuclei in the chalazal region continue to divide, however, and the spindles that are formed during these divisions tend to persist for a fairly long time.

(c) In the next stage, free nuclear divisions cease entirely and regular cell divisions begin. The endosperm enlarges to such an extent that almost all the nucellar cells are disintegrated and crushed.

(d) Finally, there is a still further increase of the endosperm tissue at the micropylar end, brought about by the divisions of the first-formed endosperm cells. This stage commences when the embryo is at about the quadrant phase (fig. 45), and the divisions are at a peak at the time of the differentiation of the cotyledons. Thus, when the embryo is passing through a highly metabolic stage and is rapidly absorbing the surrounding tissue, the endosperm cells by continuous divisions keep up a supply of nutritive tissue for the embryo.

The final result of these stages in the development of the endosperm is the formation of a massive tissue which occupies the entire seed cavity; the smaller-sized and more compactly arranged cells of the micropylar region gradually grade into the larger and considerably loosely packed cells towards the chalaza. At this stage, only one or two layers of the nucellus can be made out at the micropylar end (fig. 49), but even these become disorganized during the later stages of embryogeny.

(7) *Embryo.*—The fusion of the sperm and the egg nuclei is completed only after four to eight endosperm nuclei are formed. The zygote divides after the commencement of the second step in the endosperm development when wall formation has taken place in the micropylar region.

The first division of the zygote is transverse (fig. 43). The basal cell divides by another transverse wall to form a row of three cells (fig. 44). The cell next to the micropyle does not divide further but becomes enlarged and vacuolated (figs. 44–48); in later stages, its nucleus is slightly hypertrophied. The middle cell forms a row of

four to six suspensor cells arranged in a linear manner (figs. 45–48). The terminal cell divides longitudinally (fig. 45). Further divisions give rise to the quadrant (fig. 46) and octant stages and later a globular mass of cells is produced as shown in figures 47 and 48. This is followed by the differentiation of the cotyledons, radicle, and plumule (figs. 49–51).

During its later development the embryo digests all the endosperm so that it eventually occupies the entire cavity of the seed and is covered only by the shrivelled integuments. The cells of the embryo become loaded with starch grains and in even greater proportions with certain globular particles which take up a very deep stain with haematoxylin and osmic acid.

(8) *Pericarp.*—In the early stages, the ovary wall consists of homogeneous cells without much differentiation, but as development continues, it undergoes some interesting modifications.

Figure 55 shows a longitudinal section of a fruit at about the octant stage of the embryo. The inner four to eight layers of the ovary wall towards the micropylar region of the ovule (*c* in fig. 55; also fig. 38) represent the crystalliferous tissue whose cell walls become enormously thickened and whose cell contents become replaced by a single large polygonal crystal. In the walls of these cells develop innumerable ramified pits (fig. 56) which are continuous with those of the adjacent walls. The probable function of these layers is to afford protection to the developing embryo in addition to that provided by the two woody bracts.

The cells lying next to the crystalliferous tissue become elongated in the direction of the long axis of the ovary (*b* in fig. 55). They lose their protoplasts and the walls develop spiral thickenings (fig. 57). Ultimately the whole of this tissue assumes a spongy texture and the radicle of the embryo lies in close contact with it. Probably it serves to absorb and store moisture during the germination of the seed.

(9) *Abnormalities.—Double nucelli.* The material gathered from one particular tree in a *Casuarina* plantation had invariably two nucelli in its ovules (fig. 52). These nucelli were of unequal sizes, although both occurred within common integuments. In each nucellus the development proceeds as described for normal ovules, but the number of embryo sacs formed in the larger nucellus varies between eight and twelve, whereas in the smaller nucellus it ranges from five to eight. After the embryo sac in one nucellus has been fertilized, the other nucellus degenerates.

Supernumerary pollen tubes. Though many pollen grains germinate on the stigma, as a rule only one pollen tube succeeds in reaching the

nucellus. Only exceptionally did more than one pollen tube enter the same nucellus (figs. 59–61) and the maximum number observed was three. All the pollen tubes enter the nucellus through the chalaza, i. e., by the chalazogamous method typical of the genus. In fig. 59, showing three pollen tubes, their distal portions have become entangled over the micropylar end of an embryo sac, perhaps as a result of competition (see also fig. 59 *a*). However, no healthy or normal sexual nuclei were noticed in any of them.

In figure 60 the first pollen tube has fertilized the uppermost embryo sac in the nucellus and the endosperm is already at the four-nucleate stage. A second pollen tube in the same nucellus has reached the apex of another embryo sac but has not yet discharged its contents and the two sperm nuclei are clearly visible at its tip.

Polyembryony. Occasionally when two embryo sacs are fertilized there is a possibility of polyembryony. Figure 61 shows two pollen tubes associated with two embryo sacs, both with free nuclear endosperm. It may be inferred that seeds formed from such ovules may have two embryos.

Some ovules had two two-celled proembryos in the endosperm (fig. 53). Since there was no evidence of the entry of more than one pollen tube, the exact nature of the origin of such plural embryos remains undetermined.

In another ovule represented in figure 54, the sexually produced embryo consists of a basal cell, four suspensor cells, and the terminal embryonal mass. On the right side of this embryo, another cell, probably derived from the nucellus, has pushed into the endosperm and has undergone some free nuclear divisions. There is a possibility that this might later develop into an asexual embryo. However, this view is open to some doubt, since nucellar embryos are not known to develop from a free-nucleate cell.

A study of germination of the seeds collected from some of the *Casuarina* plantations showed two or rarely three polyembryonate seeds in every hundred. As a rule, not more than two seedlings were obtained from any seed and one of them was always smaller than the other.

Parthenocarpy. In one isolated female tree (with no other plant of the same species lying within a two mile radius of it), the development seemed to get arrested at the megaspore tetrad or two-nucleate embryo sac stage; yet fruits formed on the tree and appeared quite normal. A histological examination showed that these fruits were very similar to those from other trees but germination tests of the seeds gave only negative results. It is possible that the parthenocarpic development of the ovary is induced by the germination of

foreign pollen but the ovules fail to produce embryos owing to the lack of fertilization.

Placenta. Certain ovules showed an abnormal development of the placental tissue in comparison with the size of the two ovules (fig. 62; compare the normal ovary shown in fig. 37). The cells of such abnormal placentae were found to be devoid of cytoplasm and their walls had numerous bordered and semibordered pits (fig. 62 *a*). Fertilization followed by embryo formation up to the octant stage was seen to take place normally in the ovules developed on such placentae but no further stages could be followed and the significance of this behavior of the placenta remains to be studied.

Casuarina montana

The seeds of this species were obtained from Malaya in 1940. They were germinated and the seedlings transplanted to a place isolated from the *C. equisetifolia* plants. The trees began to bear flowers from the fourth year onwards and produced viable seeds. The following observations are based on material collected from the above plants.

(1) *Ovary and ovules.*—The ovary is bicarpellary but only one of the carpels is fertile. This contains two ovules. During early stages, their axes are more or less at right angles to the longitudinal axis of the ovary (fig. 63), but later become so oriented that the micropyle points towards the style (fig. 64). On the side towards the placenta, the outer integument is fused with the placental tissue to form the bridge which serves for the passage of the pollen tube from the base of the style to the chalaza.

(2) *Megasporogenesis and female gametophyte.*—The archesporium differentiates as a group of three or four hypodermal cells, each of which divides periclinally to form the primary wall cells and the primary sporogenous cells. The former divide to give rise to seven or eight wall layers; the latter divide to form a massive sporogenous tissue.

A point of special interest in this species is that while the sporogenous cells in the upper part of the nucellus are differentiating into megaspore mother cells, those in the lower part continue to divide actively for some time, adding to the sporogenous tissue. Proceeding from the basal part upwards, one can see a series of stages in megasporogenesis and megagametogenesis in the same ovule (fig. 66).

The extension of the procambial strand of the funiculus into the chalaza and thence into the nucellus takes place in the same way as described for *C. equisetifolia* (figs. 63–66). The cells of the procambial strand lying within the nucellus do not become tracheidal at any

stage of development but completely degenerate while the embryo sac caeca are developing.

The megaspore mother cell enlarges and elongates appreciably before undergoing the meiotic divisions. A linear tetrad of megaspores is formed. All the megaspores show a tendency towards further development to varying extents, although occasionally one or more of them degenerate at an early stage. The mature embryo sac has the usual organization except that the antipodals are ephemeral. The synergids sometimes have an egg-like appearance with apical vacuoles and basal nuclei (fig. 68). The polar nuclei fuse before fertilization and the secondary embryo sac nucleus lies just below the egg apparatus (fig. 67).

Only from three to five embryo sacs in a nucellus usually attain a fertilizable condition, the rest degenerating at an earlier stage. Embryo sac caeca are formed as in *C. equisetifolia.*

The structure of the ovule at the time of fertilization is interesting in this species. The nucellus pushes itself beyond the integuments through the micropylar end and comes to lie exposed in the ovary (figs. 65, 66). This situation is a result of the prolonged divisions of the primary archesporial cells towards the chalaza.

(3) *Fertilization.*—The pollen grains are two-celled at the time of shedding and pollination is accomplished by wind during the months of September and October. As in *C. equisetifolia*, the pollen tube enters the chalaza through the bridge and sends out numerous blind branches at the chalaza (figs. 69–71), some of which show a tendency to pass out to the superficial cell layers of the ovule.

The nucleus of the generative cell divides in the pollen tube into two sperms. They are surrounded by cytoplasmic sheaths (figs. 71, 72) but no trace of a cell wall or limiting membrane could be made out. The sperms are always associated with the tube nucleus (figs. 70–73).

The vegetative nucleus and the sperms do not enter any of the blind branches of the pollen tube but remain in its subterminal part and eventually pass into the particular branch which grows up into the nucellus. Here, the lumen of the tube expands and the wall thickens slightly. After passing between the antipodal caeca of the embryo sacs, the pollen tube reaches the micropylar tip of one of the sacs. At this point the pollen tube pierces the embryo sac and discharges its contents into it. Actual stages in double fertilization were not seen. The earliest stage observed after fertilization (fig. 76) showed the course and the remains of the pollen tube, the zygote and four endosperm nuclei. The persisting synergid had two nuclei.

Many pollen tubes show abundance accumulation of starch grains while they traverse the chalaza and nucellus.

(4) *Abnormalities.*—Figure 74 represents a pollen tube which behaved in an abnormal manner. After entering the nucellus, it passed out of the nucellus and then recurved toward the chalaza, the tip of the tube penetrating between the inner integument and the nucellus. The tip of this pollen tube contained the sperms and the vegetative nucleus (fig. 72). In the nucellus of this ovule, one embryo sac had attained maturity but degeneration had already set in.

Another abnormality relates to a peculiar branching of the pollen tube after it had reached the top of an embryo sac. It gave rise to many short branches which hung on the top of the embryo sac in a drooping manner. One of the branches contained the vegetative nucleus and sperms (fig. 73). It is interesting to note that before reaching the micropylar end of the embryo sac, this pollen tube had wound around the embryo sac (fig. 75), which was itself in a state of degeneration.

Chromosome Number

In the cells of the root tips of both species studied at present eighteen chromosomes were seen (figs. 58, 77).

Comparisons with Other Species of Casuarina

(1) *Microsporogenesis.*—The present study is the first to give an account of the development of pollen in *Casuarina.* The archesporium differentiates from a group of hypodermal cells. The primary parietal cells produce the endothecium, one or two middle layers, and a tapetum. The primary sporogenous cells function directly as the microspore mother cells, which give rise to microspores. The pollen grains are shed at the two-celled stage.

(2) *Ovary and ovules.*—The wing-like expansion of the ovary, the crystalliferous and sclerosed cells occurring in its inner cell layers, and the lateral origin of the epitropous ovules from the placenta are features common to all species so far investigated. Attention may however, be drawn here to the figures 6, 7 and 8 of Poisson (1871), illustrating a few stages in the development of ovules in *C. equisetifolia.* A study of these figures give the impression that the two ovules originate from the base of an unilocular ovary although Poisson does not refer to this point in the text. Actually, the ovules arise laterally from the placenta and become erect during their subsequent growth, as is demonstrated in the present paper.

The two integuments arise more or less simultaneously. The micropyle is erect in all species excepting *C. equisetifolia,* in which

sometimes it is slightly bent or curved. In *C. montana*, a micropyle is absent during later stages, as the upper part of the nucellus pushes itself out of the two integuments and comes to lie exposed in the ovary at the time of fertilization.

(3) *Conducting strand.*—A procambial conducting strand becomes organized in the chalazal region of the ovule in both the species studied by me. This continues upwards into the nucellus as a central core, surrounded at its tip by the sporogenous cells. Some of the latter themselves seem to elongate and partially contribute to the extension of the strand, instead of functioning as megaspore mother cells. However, the cells were not seen to become tracheidal at any time of their development, although those outside the nucellus developed the usual spiral thickenings by the time of fertilization.

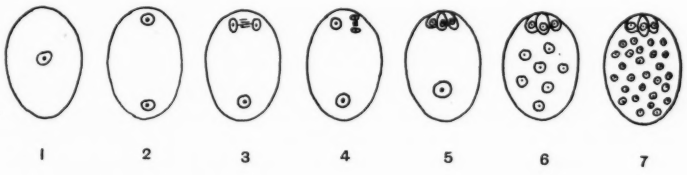
A detailed study of Treub's illustrations seems to indicate that a similar conducting strand is also present in the species investigated by him. His plate 16, figures 1–4 show beyond doubt that the conducting strand extends up to the base of the nucellus. He reports that in *C. glauca* and *C. Rumphiana* some of the archesporial cells themselves become transformed into tracheids (see his plate 19, figures 2, 3, 5; and plate 20, figures 2, 5, 6, 7). In other species, however, viz. *C. suberosa* (Treub, 1891), *C. stricta* (Frye, 1903), *C. equisetifolia*, and *C. montana* (present study), such a transformation seems to be absent.

(4) *Sporogenous tissue.*—In *C. suberosa*, according to Treub, several hypodermal cells ("primary mother cells") begin dividing periclinally and become buried in the nucellus; the limits of this tissue are more clearly defined towards the micropyle and the sides but not towards the chalaza. In this region, owing to the presence of a strong "intercalary growth", Treub was induced to suspect that the lower portion of the sporogenous tissue arose secondarily from cells which do not belong to the primary archesporium.

Frye (1903) noted that in *C. stricta*, the cells of the hypodermal archesporial tissue divide periclinally to give rise to the primary wall cells and primary sporogenous cells. He did not observe any "intercalary growth" at the base of the nucellus and considered that there was no other method of the origin of the sporogenous tissue than from the primary archesporium. Juel's (1903) work on *C. quadrivalvis* and the present study on *C. equisetifolia* confirm this. *C. montana* is similar but has another interesting feature in that the sporogenous tissue continues to increase in bulk by the intercalary divisions of the primary sporogenous cells themselves, so much so that the major portion of the nucellus becomes pushed out of the integuments.

Perhaps a similar phenomenon, to a lesser degree, exists in *C. suberosa*, which led Treub to suspect that some of the sporogenous cells situated towards the chalaza might have arisen from cells other than those belonging to the primary archesporium.

(5) *Female gametophyte.*—According to Treub, the primary chalazal nucleus of the binucleate embryo sac remains at rest for some time while the other divides immediately into two. Frequently one of these divides again so that a group of three nuclei is formed at the micropylar end. These become surrounded by cytoplasm and individual cellulose membranes and constitute the egg apparatus. After this, the primary chalazal nucleus is said to undergo a number of free nuclear divisions, resulting in as many as 50 nuclei, which constitute the endosperm. It is at about this stage that the embryo sac is said to be ready for fertilization. The gist of his observations is diagrammatically represented in the figure below:



Several of the embryo sacs developing in a nucellus may send out chalazal prolongations. Only one of them (the "fertile macrospore") is, however, said to develop the egg apparatus and endosperm nuclei and attain a fertilizable condition. The other embryo sacs ("sterile macrospores") do not develop to this stage, being devoid of an egg apparatus but having one or two nuclei.

Other unusual features mentioned by Treub are the absence of antipodals and the anomalous position of the egg apparatus, frequently seen at a considerable distance below the apex of the embryo sac.

Juel (1903) studied *C. quadrivalvis* but his observations do not go beyond the tetrad formation. Frye (1903) made a more detailed study which, together with my own observations, enable us to draw the following conclusions:

(i) There is a linear tetrad of megaspores formed and usually more than one megaspore of the tetrad continues further development.

(ii) The embryo sac is of the monosporic eight-nucleate type and

consists of an egg apparatus (one egg and two synergids), two juxtaposed polar nuclei and three ephemeral antipodal nuclei.

(iii) Of the many enlarging megaspores in a nucellus, as many as a score or so develop into normally organized embryo sacs. Others become arrested in their development. Treub's distinction of the embryo sacs into "fertile" and "sterile" is incorrect and misleading. As a rule, it is one of the two or three embryo sacs that are situated in the upper part of the nucellus which becomes fertilized.

(iv) Almost all the embryo sacs in a nucellus form the antipodal caeca.

(v) Endosperm formation does not occur in unfertilized embryo sacs.

(6) *Course of the pollen tube.*—The most remarkable feature in *Casuarina* is the chalazogamous route taken by the pollen tube to reach the egg apparatus. Treub was the first to discover this phenomenon and his description may be briefly stated as follows.

The pollen tube proceeds from the style into the bridge which joins the upper part of the ovule with the placenta in continuation of the style. From here, it proceeds downwards to the end of the vascular bundle which terminates in the chalaza. After giving out one or two short branches, it now continues onward into the chalaza and the nucellus, finally passing up to the embryo sacs. The passage of the pollen tube into the nucellus is facilitated by the "tails" of the macrospores which have digested much of the tissue lying in its path in the chalaza.

In referring to the entry of the pollen tube into the embryo sac, Treub repeatedly says that the tip of the pollen tube does not apply itself against the embryo sac membrane at the point where the egg apparatus is situated but at the opposite end, suggesting thereby that it discharges its contents into the embryo sac somewhere at the basal end of the embryo sac. Frye's observations on the pollen tube course in *C. stricta* do not throw more light on the matter. He agrees that the pollen tube enters the chalaza through the bridge and that it gives out some branches in this region, but he is not definite about its further behavior and writes, "I doubt whether there is any fixed path within the nucellus for the tube."

In this respect my observations on *C. equisetifolia* and *C. montana* may be summarized as follows:

As in the previously investigated species of *Casuarina*, the pollen tube enters the base of the ovule by passing through the bridge. Upon entering the chalaza, it invariably sends out branches. That branch which contains the sperm nuclei proceeds upward into the nucellus through the spaces *between* the vesicular swellings of the

embryo sac caeca and finally reaches the micropylar end of an embryo sac (usually occupying the uppermost position in the nucellus). No pollen tube was seen to enter at the basal end of an embryo sac and travel *through* its cavity.

Regarding the attachment of the pollen tube to the wall of the embryo sac, Treub emphasizes that this occurs at a point far away from the egg apparatus. In the present investigation, however, the pollen tube always was seen to implant itself on the embryo sac only at the point of situation of the egg apparatus, which is organized always at the micropylar end of the sac.

It will not be out of place here to call attention to some incorrect versions of Treub's work. Thus, Oliver (1895) writes: "From the base of the ovule *the pollen tube penetrates towards the apex of the ovule, reaching the egg cell from below*, not from above as in the cases in which the pollen tube entered the ovule by the micropyle". Coulter and Chamberlain (1903), in referring to figure 2, plate 22[3] of Treub's paper (their fig. 67 a) remark that the pollen tube "becomes associated with the numerous elongated sterile megaspores; and doubtless they are of service in rendering the passage easy; *later it enters the antipodal region of the embryo sac and approaches the egg apparatus from that direction*". Willis (1931) observes that "it (the pollen tube) passes upwards *inside a sterile macrospore* and finally enters the fertile one." Rendle (1938) also speaks of the pollen tube as ". . . . often passing up the caecum of an embryo sac."[4]

A careful perusal of Treub's writing shows, however, that nowhere does he speak of the pollen tube actually entering an embryo sac through its basal end and travelling inside its cavity to discharge its contents below the egg apparatus. As has already been stated, according to him, the pollen tube touches the embryo sac at a place far away from the place of insertion of the egg apparatus and discharges its contents from such a point of contact, so that the male gametes will have to travel a considerable space inside the embryo sac in order to reach the egg and polar nuclei.

(7) *Branching of the pollen tube.*—It is a very common feature in the genus *Casuarina* to find the pollen tube branching in the chalazal region of the ovule. Treub demonstrated that some tubes formed short branches even during their course between the bridge and the

[3] According to Treub, this figure merely represents the pollen tube lying below the embryo sac, which overlaps it. But Coulter and Chamberlain (1903, p. 149) contend that the pollen tube is lying within the embryo sac. See Treub's figures on plate 27, figures 1 *a–h*, which clearly convey his version.

[4] The italics in this paragraph are mine.

chalazal region and also in the nucellus. In the present investigation it is seen that in *C. equisetifolia*, the pollen tube may branch even in the stigma. In *C. montana*, one tube was observed which had coiled around an embryo sac and formed a tuft of short branchlets.

(8) *Fertilization.*—Treub's account may be summarized as follows:

a. Besides the egg, the mature embryo sac contains as many as 50 endosperm nuclei before fertilization.

b. The pollen tube implants itself on the membrane of the embryo sac at a point far away from the place of attachment of the egg apparatus.

c. A male nucleus penetrates the membrane of the pollen tube and that of the embryo sac at the point of mutual contact, enters the embryo sac, and traverses the greater part of the sac and ultimately fuses with the egg nucleus after entering the egg cell from below.

Frye's account for *C. stricta* showed, however, that the mode of fertilization is quite similar in most ways to that described for other angiosperms. The present investigation fully confirms this. Briefly, the mature embryo sac is eight-nucleate and does not have any endosperm nuclei at the time of fertilization; the tip of the pollen tube pierces the embryo sac membrane at the point of situation of the egg apparatus; of the two sperms discharged by the tube, one fuses with the nucleus of the egg and the other with the two polar nuclei; triple fusion and the subsequent divisions of the primary endosperm nucleus commence before syngamy is completed; and the zygote divides only after wall formation starts in the endosperm. It seems certain (see also Frye, 1903) that Treub mistook a fertilized embryo sac for an unfertilized one and a discharged pollen tube for one that had not yet opened. Further, the single nucleus which Treub occasionally saw in the pollen tube was probably the undischarged and degenerating vegetative nucleus.

(9) *Endosperm and embryo.*—The observations made in the present study that originally the endosperm is nuclear and that cell formation proceeds from the micropylar end towards the chalaza are in agreement with Treub's findings. Some additional points noted by me are that the endosperm completely replaces the nucellus and that the embryo consumes all of this tissue.

In *C. equisetifolia* the first wall laid down in the zygote is transverse. By another transverse division of the basal cell, a row of three cells is organized. The micropylar cell and its nucleus enlarge but do not divide further; the middle cell forms a suspensor of four to six cells and the terminal one develops into the embryo proper. This roughly corresponds to Schnarf's (1929) "cruciferous type" or to one of its variants.

A Brief Resume of Chalazogamy

In drawing comparisons between the Casuarinaceae and other chalazogams, one naturally turns to the so-called Amentiferae, a group which is generally considered to comprise the following families: Betulaceae, Fagaceae, Juglandaceae, Corylaceae, Myricaceae, Salicaceae, Leitneriaceae, Balanopsidaceae, and Garryaceae.[5] Of these, only Betulaceae (*Betula, Alnus*), Corylaceae (*Corylus, carpinus, Ostrya*) and Juglandaceae (*Juglans, Carya, Pterocarya*) are known to exhibit chalazogamy (see table 1).

TABLE 1

Families and species	Type of chalazogamy	Author and reference
1. BETULACEAE		
Betula alba	Extravesicular chalazogamy	Nawaschin, 1894
B. pendula ?	Extravesicular chalazogamy	Nawaschin, 1894
Alnus glutinosa	Extravesicular chalazogamy	Nawaschin, 1894
A. viridis	Extravesicular chalazogamy	Nawaschin, 1899
2. CORYLACEAE		
Corylus avellana	Intravesicular chalazogamy	Benson, 1894 Benson et al., 1906 Nawaschin, 1895 Nawaschin, 1899 a
Carpinus betulus	Intravesicular chalazogamy	Benson, 1894 Benson et al., 1906
Ostrya carpinifolia	Not ascertained	Finn, 1936
3. JUGLANDACEAE		
Juglans cinerea	Extravesicular chalazogamy	Nawaschin, 1895 a
J. regia	Extravesicular chalazogamy	Nawaschin, 1895 a Nast, 1941 Nawaschin & Finn, 1913
J. nigra	Extravesicular chalazogamy	Nawaschin & Finn, 1913
Carya olivaeformis	Not ascertained	Billings, 1903
C. pecan	Not ascertained	Woodroof & Woodroof, 1927
C. alba	Not ascertained	Karsten, 1902
C. cordifolia	Not ascertained	Karsten, 1902
Pterocarya Fraxinifolia	Not ascertained	Karsten, 1902
4. CASUARINACEAE		
Casuarina suberosa	Extravesicular chalazogamy	Treub, 1891
C. glauca	Extravesicular chalazogamy	Treub, 1891
C. Rumphiana	Extravesicular chalazogamy	Treub, 1891
C. stricta	Not ascertained, but extravesicular chalazogamy is most probable	Frye, 1903
C. equisetifolia	Extravesicular chalazogamy	(Present study)
C. montana	Extravesicular chalazogamy	(Present study)

[5] A suggestion has been made (see Hallock, 1930) that the Garryaceae should be removed from this group and placed among the Umbelliflorae.

(1) *Types of chalazogamy.*—In the Betulaceae, Juglandaceae, and Casuarinaceae, the pollen tube enters the nucellus through the intercellular spaces and reaches the top of the embryo sac; from there it bends down and applies itself against the membrane of the embryo sac near the egg apparatus in the same way as if it had entered through the micropyle (figs. 37, 38). On the other hand, in the Corylaceae, according to Benson (1894), the pollen tube comes in contact with the caecum of the embryo sac that is to be fertilized, pierces it and proceeds upwards through its cavity, discharging its contents just below the egg (see Benson, 1894, figs. 50, 51; Benson et al. 1906, fig. 1).

For the first type, which occurs in the families Betulaceae, Juglandaceae, and Casuarinaceae, the name "extravesicular chalazogamy" may be applied, implying that the pollen tube reaches the egg by travelling outside the embryo sac that is to be fertilized. For the second type, which is reported in the Corylaceae, the term, "intravesicular chalazogamy" may be used, from the fact that here the pollen tube reaches the egg by a direct penetration of the embryo sac at its chalazal end.

(2) *Branching of the pollen tube.*—Table 2 presents a list of those plants that are characterized by having branching pollen tubes. It may be noted from a comparison of tables 1 and 2 that branching tubes occur in almost all chalazogamous species.

TABLE 2

Plant	Author	Remarks
Fagus sp.		Benson considers this to be a very general feature in the "Amentiferae." In *Quercus*, the pollen tubes form short branches at their tip.
Quercus sp.		
Betula alba	Benson, 1894	
Carpinus betulus		
Corylus avellana		
Betula alba	Nawaschin, 1894	After reaching the apex of the embryo sac, the tip of the pollen tube gives rise to short protuberances.
Juglans regia	Nawaschin, 1895 a	The pollen tubes profusely branch throughout their course.
Juglans nigra	Nawaschin & Finn, 1913	
Carya olivaeformis	Billings, 1903	As in *Juglans*.
Ulmus americana	Nawaschin, 1898	As in *Juglans*.
Cannabina sativa	Zinger, 1898	The tube gives rise to short branches at its tip.
Pothos longifolia	Hofmeister, 1859	The tube proliferates into short branches.
Hippeastrum aulicum		
Casuarina species	Treub, 1891 Frye, 1903 (Present study)	Branching invariably occurs at the chalazal region of the ovule; occasionally also in the stigma and in the nucellus.

(3) *Hibernation.*—The period between pollination and fertilization is usually a long one in the chalazogams. In most genera, the ovules

do not have even the archesporial tissue differentiated at the time of pollination. This has led some writers (e. g. Baillon, cited by Benson, 1894) to think that here the pollen tube undergoes a period of rest in an encysted form and that it may even pass into a "secondary pollen grain" stage. Benson was, however, unable to obtain any evidence for the existence of such a condition.

I made a special effort to study this point in *C. equisetifolia.* Collections were made on every third day after pollination and the pistils were examined both with the help of suitably cleared whole mounts as well as serial sections. No pollen tube showed evidence either of cyst formation or of any other similar adaptation for undergoing a period of rest. In this connection the following data speak for themselves.

a. The pollen grains germinate on the stigma within three days after pollination. At this stage, the ovules are still very young and only beginning to grow. b. About six days later, when the pollen tube is crossing the bridge, the nucellus shows a differentiation of sporogenous and parietal layers. c. Twelve days later, when the pollen tube has already reached the chalaza, the embryo sacs are still at the two- or four-nucleate stages. d. Twenty days after the preceding stage, the pollen tube begins to branch in the chalaza and mature embryo sacs are seen towards the upper part of the nucellus. e. Fertilization occurs from about nine to twelve days after the stage d. The duration between pollination and fertilization is therefore, about 45–50 days and the development of the pollen tube continues without any break for the entire period.

(4) *Other sporogamous methods of the pollen tube.*—True chalazogamy—in which the pollen tube enters the ovule through the chalaza and reaches the micropylar end of the embryo sac by an upward movement in the nucellus—has till now been reported only in the Casuarinaceae, Corylaceae, Juglandaceae, and Betulaceae. Other aporogamous modes of the pollen tube behavior are also known, however. Generally in such plants (*Ulmus,* Nawaschin, 1898; *Cannabis,* Zinger, 1898; *Cucurbita,* Longo, 1901; *Alchemilla,* Murbeck, 1901) the pollen tube bores through the integuments at some phase of its course in order to enter the nucellus; moreover, the final entry will be invariably from a place nearer to the micropyle. It may also be noted that such abnormal behavior is not constant in any of the species and is variable even in the different ovules. On the other hand, in all the chalazogamous plants, the pollen tubes invariably pass from the chalaza to the egg apparatus through the nucellus (Casuarinaceae, Betulaceae, Juglandaceae) or through the very embryo sac that is to be fertilized (Corylaceae).

(5) *Terminology.*—Some authors (e. g. Rendle, 1938) consider the aporogamous behavior of the pollen tube also as a kind of chalazogamy. This view does not seem to be correct. Wettstein (1924), Porsch (1907), Nawaschin & Finn (1913) and others have already distinguished true chalazogamy from aporogamy. Pirotta & Longo (1900) proposed the term "acrogamy" for the entrance of the pollen tube through the micropyle; "basigamy" for its entrance through the chalaza; and "mesogamy" for its entrance by intermediate routes. In the opinion of the present student, the term "chalazogamy" should be retained in place of "basigamy" on the principle of priority. "Porogamy" which denotes the entrance of the pollen tube through the micropyle has been in use for a very long time and there is no need to replace it by a new term, "acrogamy." The term "mesogamy" is suitable for all cases of an intermediate nature.

Summary

Two species of *Casuarina, C. equisetifolia* and *C. montana,* are studied from the point of view of embryology.

In the microsporangium the primary parietal layer gives rise to the endothecium, middle layers, and tapetum, and the primary sporogenous layer to the microspore mother cells. The latter undergo simultaneous divisions to form microspore tetrads. The pollen grains are two-celled at the time of shedding.

The ovary is made up of two locules of which one is sterile and the other bears two ovules. The ovules originate from the placenta in a lateral manner but later become epitropous, with the micropyle pointing towards the style. The chalazal conducting strand continues upwards into the nucellus. Some of the centrally situated sporogenous cells also seem to elongate and contribute towards the extension of the strand in the nucellus.

The primary sporogenous cells in the nucellus divide mitotically to produce an increased number of megaspore mother cells during the same time as the primary parietal layer is giving rise to the parietal tissue.

The female gametophyte is of the monosporic eight-nucleate type. More than one megaspore of a tetrad germinates further so that as many as 20–25 embryo sacs are present in the same nucellus. The embryo sac develops a tubular chalazal caecum; the antipodals are ephemeral.

The pollen tube enters the chalaza and undergoes some branching. The particular branch carrying the gametes makes its way between the swollen vesicles of the embryo sac caeca and eventually reaches the

top of an embryo sac. Entry into the embryo sac is effected at a point close to the egg apparatus.

Early divisions of the endosperm are free nuclear. Wall formation commences from the micropylar end, while free nuclear divisions still continue at the antipodal end.

The embryo development roughly corresponds to the "cruciferous type." Occasionally polyembryony occurs, which may be due to the entry of more than one pollen tube followed by a fertilization of two or more embryo sacs in the same nucellus.

A brief survey of chalazogamous plants with reference to pollen tube behavior is presented. Certain terminologies connected with chalazogamy are discussed.

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EXPLANATION TO FIGURES

PLATE I. *Casuarina equisetifolia*

Fig. 1. Side view of the male flower; the outer perianth towards the side of the onlooker is partly trimmed off, × 40.

Fig. 2. Side view of the same, from the adjacent side, × 40.

Fig. 3. Floral diagram of the male flower.

Fig. 4. Median longitudinal section of a young male flower, × 60.

Fig. 5. L. s. of a portion of a female strobilus showing the position of the woody valves and ovaries, × 10.

Fig. 6. A single female flower, × 10.

Fig. 7. Floral diagram of the female flower.

Fig. 8. Ovary after pollination; note the shrivelled style and the development of the wing-like expansion of the ovary, × 5.

Fig. 9. L. s. of a very young ovary showing the position and topography of the ovules, × 40.

Fig. 10. Same as Fig. 9, but still older, at about the time of fertilization. Note the erect position of the ovules and the "bridge," × 40.

Fig. 11. Same as Fig. 10; Figs. 11a to 11h represent transverse sections of an ovary at different levels as marked in Fig. 11 by corresponding letters. 11c′ and 11c″ represent the longitudinal sections passing through the planes indicated by lines c′c′ and c″c″ in Fig. 11c, × 40.

PLATE II. *Casuarina equisetifolia*

Fig. 12. T. s. of a part of a young anther to show the archesporium; two locules are shown. In one of the locules the archesporial cells are resting, in the other, in division, × 450.

Figs. 13 and 14. Differentiation of the endothecium, wall layers and tapetum, × 450.

Fig. 15. A loculus of an anther showing the epidermis, endothecium, wall layers, tapetum, and microspore mother cells, × 450.

Fig. 16. Some of the cells of the endothecium to show the characteristic banded thickenings, × 300.

Fig. 17. Uninucleate microspore, × 300.

Fig. 18. Division of the nucleus of the microspore, × 300.

Fig. 19. Pollen grain showing the vegetative and generative cells, × 300.

Fig. 20. Pollen grain at the time of shedding, × 300.

Fig. 21. L. s. of young nucellus showing three hypodermal archesporial cells, × 450.

Fig. 22. Same as Fig. 21. Slightly later stage showing the increase in the sporogenous as well as in the parietal tissue, × 450.

Fig. 23. L. s. of an entire ovule at the time of commencement of the meiotic divisions in the megaspore mother cells. Note the conducting strand extending into the core of the sporogenous cells, × 200.

Fig. 24. Division of megaspore mother cell and the formation of dyad cells, × 450.

Fig. 25. Division of the dyad cells leading to the formation of the tetrad of megaspores, × 450.

Fig. 26. A portion of the sporogenous tissue showing tetrads of megaspores, some degenerating and others germinating to various stages; note the commencement of the embryo sac caecum in the two-nucleate embryo sac, × 450.

Fig. 27. Two linear tetrads in which all the megaspores have developed into eight-nucleate embryo sacs; many of them are showing indications of internal organization, × 450.

Fig. 28. Division of the nuclei of the two-nucleate sac, × 450.

Fig. 29. Division of the nuclei of the four-nucleate sac, × 450.

Fig. 30. Unorganized eight-nucleate embryo sac, × 450.

Fig. 31. A typical eight-nucleate embryo sac at maturity, × 400.

Fig. 32. Ovule at the time of fertilization; note the embryo sac caeca, whose terminal swllings are lying in contact with the vascular conducting strand, × 60.

Figs. 33–35. Variations in the structure of the egg apparatus; explanation in text, × 450.

Plate III. *Casuarina equisetifolia*

Fig. 36. Germination of pollen grains on the stigma, × 60.

Fig. 37. Optical longitudinal section of an entire ovary to show the general topography of the internal organs and the course of the pollen tube, × 20.

Fig. 38. L. s. of an ovary reconstructed from six serial sections, to show the course of the pollen tube in the ovule, × 80.

Fig. 39. Double fertilization, × 300.

Fig. 40. Tip of a pollen tube which was on its way to an embryo sac in the nucellus; note the sheathed condition of the sperms, × 450.

Fig. 41. Embryo sac after fertilization showing the zygote and eight endosperm nuclei in division, × 100.

Fig. 42. An early stage in the formation of endosperm; note the peripheral arrangement of the nuclei, × 100.

Fig. 43. Embryo sac showing two-celled embryo; the endosperm is becoming cellular in the micropylar region and free nuclear divisions are still taking place in the chalazal region. Note the persistence of spindles in the lower half, × 60.

Plate IV. *Casuarina equisetifolia*

Fig. 44. Portion of endosperm showing the three-celled proembryo, × 200.

Fig. 45. Embryo showing the haustorial cell, four suspensor cells and the longitudinally divided terminal cell. Note the divisions in the endosperm cells, × 140.

Fig. 46–48. Further stages in the development of the embryo (Fig. 48 is a drawing of a whole mount preparation of a dissected embryo), Figs. 46, 47, × 315; Fig. 48, × 275.

Fig. 49. Later stage of the embryo, in which the cotyledons have differentiated; a few of the nucellar cells are still visible below the micropyle, × 30.

Fig. 50. L. s. of a late embryo showing cotyledons, radicle, and the stem tip, × 40.

Fig. 51. An embryo (surface view of a cotyledon) at the stage of dispersal, × 30.

Fig. 52. Ovule with double nucelli, × 400.

Fig. 53. Two two-celled proembryos surrounded by endosperm cells, × 450.

Fig. 54. A sexually produced embryo and a multinucleate nucellar cell which perhaps develops into an adventive embryo, × 275.

Plate V. *Casuarina equisetifolia*

Fig. 55. L. s. of a fruit at the time of the octant stage of the embryo, × 20. *a*. Stylar tissue; *b*. Pad of spirally thickened cells; *c*. Crystalliferous tissue; *d*. Outer integument; *e*. embryo; *f*. Nucellus; *g*. Endosperm; *h*. inner wall of the ovary; *i*. Ovular stalk.

Fig. 55a. T. s. at the region dotted in Fig. 55, × 20.

Fig. 56. Crystalliferous cells from the region *c* in Fig. 55, showing ramified pits and the solitary crystal in each cell, × 450.

Fig. 57. Spirally thickened cells from region *b* in Fig. 55, × 100.

Fig. 58. A metaphase plate from a cell of the root tip, showing 18 chromosomes, × 900.

Fig. 59. Ovule in which three pollen tubes have entered the nucellus and their tips are entangled over an embryo sac, × 200.

Fig. 59a. Tangled portion of the pollen tubes enlarged from Fig. 59, × 300.

Fig. 60. Ovule in which one pollen tube has fertilized an embryo sac and a second pollen tube is lying on the apex of another embryo sac, × 200.

Fig. 61. Ovule in which two pollen tubes have fertilized two embryo sacs, × 200.

Fig. 62. Ovule with abnormal placenta, × 40.

Fig. 62a. Cells from the abnormal placenta showing pits (enlarged from fig. 62), × 450.

Plate VI. *Casuarina montana*

Fig. 63. L. s. of a young ovary showing the horizontal position of young ovules, × 20.

Fig. 64. The same, later stage showing the epitropous position of the ovule, × 20.

Fig. 65. Young nucellus, just after the formation of the parietal layers; note the sporogenous cells still dividing towards the lower end, × 275.

Fig. 66. A later stage of the nucellus showing the germination of the megaspores, the procambial strand in the nucellus and the divisions of the sporogenous cells still going on towards the basal end, × 275.

Fig. 67. Mature embryo sac showing the egg apparatus and secondary embryo sac nucleus; the antipodals are degenerating in this sac, × 450.

Fig. 68. Egg apparatus showing the egg cell and two synergids, × 450.

PLATE VII. *Casuarina montana*

Fig. 69. L. s. of an ovule showing the entry of the pollen tube and its branching in the chalazal region, × 20.

Fig. 70. Branching portion of the pollen tube enlarged from Fig. 69 to show the sperms and the vegetative nucleus, × 100.

Fig. 71. A portion from Fig. 70 further enlarged to show the alignment of cells in the chalaza and the details of the sperms and vegetative nucleus, × 450.

Fig. 72. Sperms and the vegetative nucleus enlarged from the tip of the pollen tube in Fig. 74, × 450.

Fig. 73. Sperms and the vegetative nucleus enlarged from one of the branchlets of the pollen tube in Fig. 75, × 450.

Fig. 74. An abnormal behavior of the pollen tube, × 400.

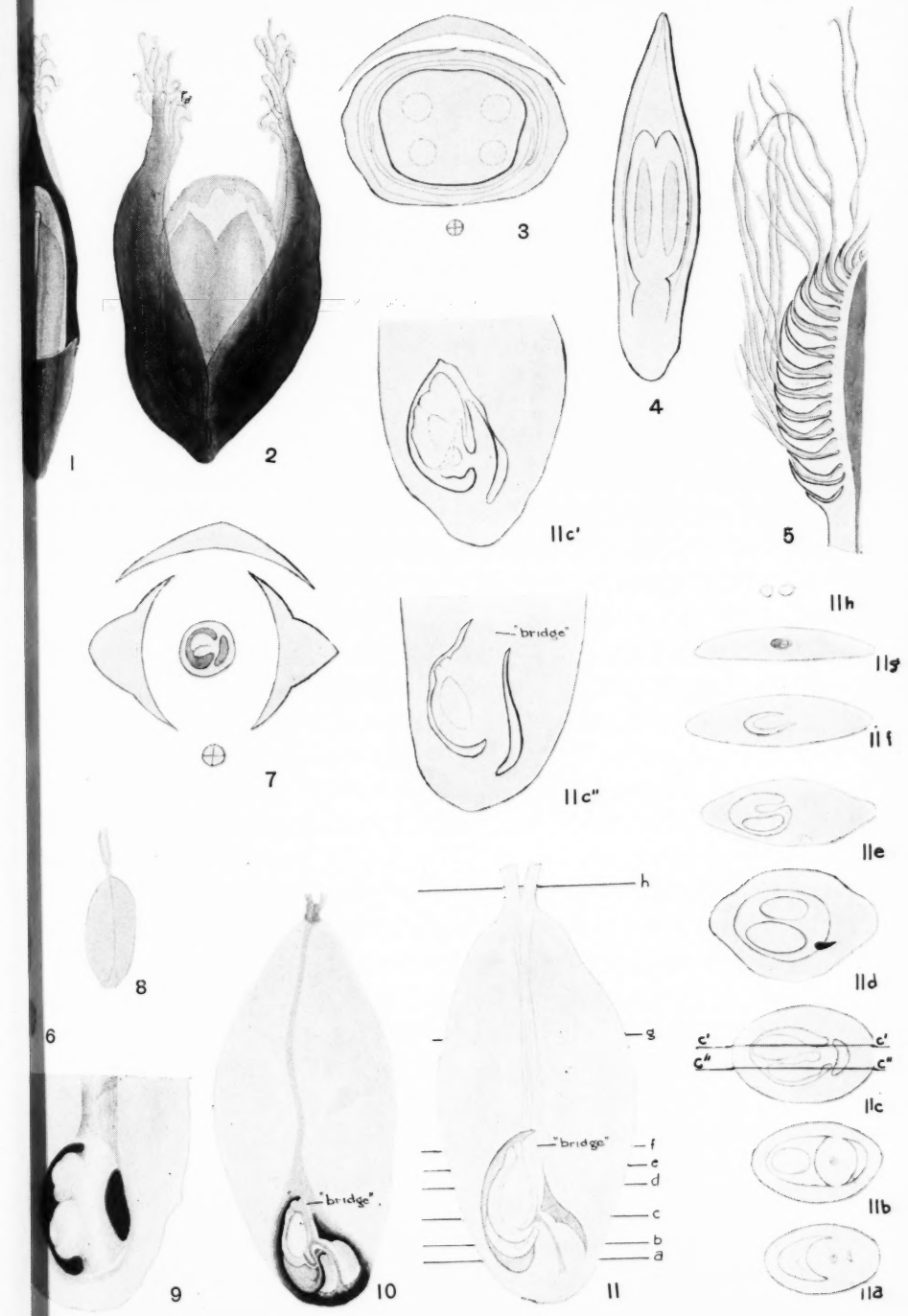
Fig. 75. Another abnormal behavior of the pollen tube; explanation in text, × 100.

Fig. 76. A fertilized embryo sac showing the course of the pollen tube in the nucellus, zygote and four endosperm nuclei; the persisting synergid in this case is binucleate, × 300.

Fig. 77. A metaphase plate from a cell of the root tip, showing 18 chromosomes, × 900.

PLATES

PLATE 1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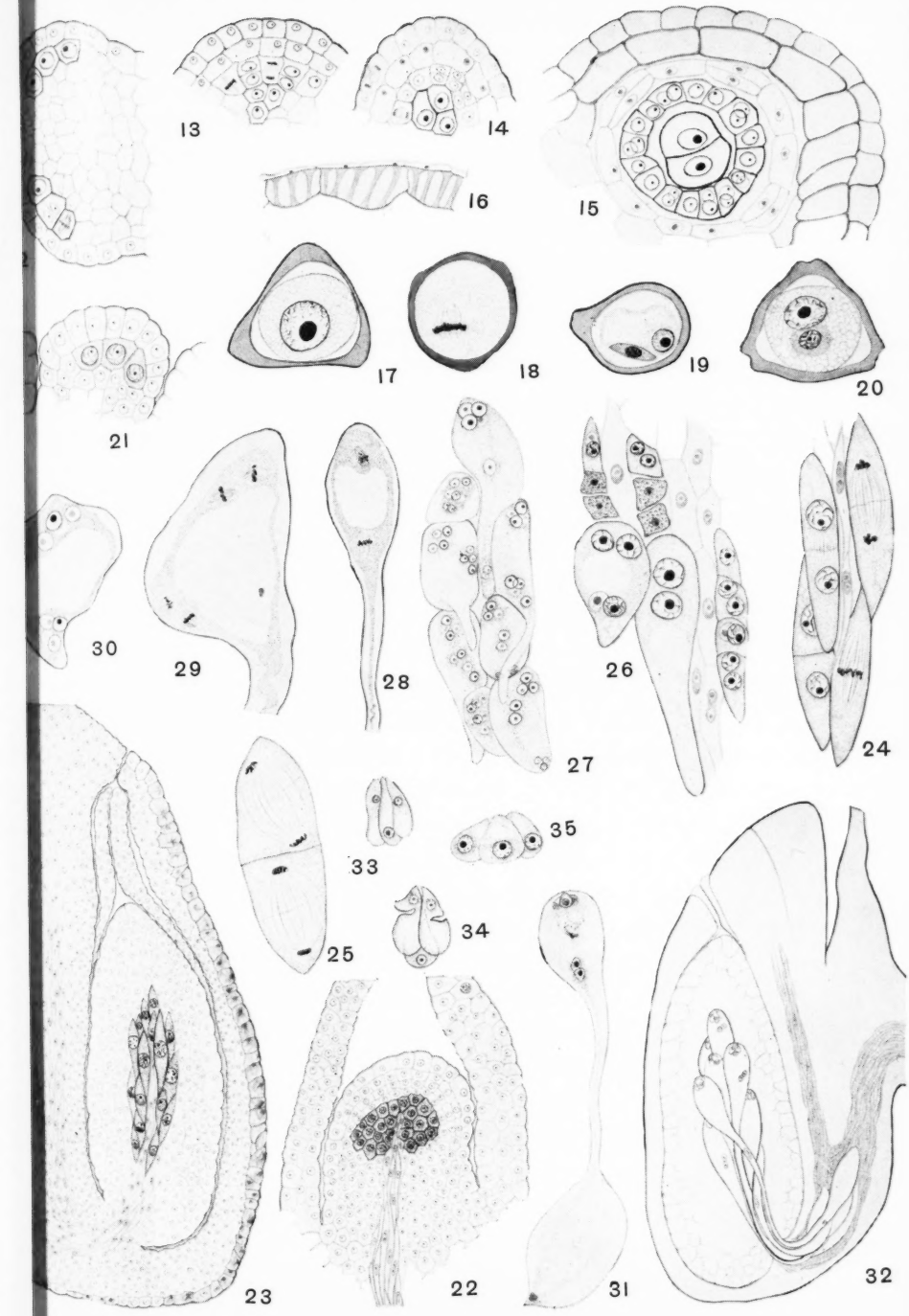

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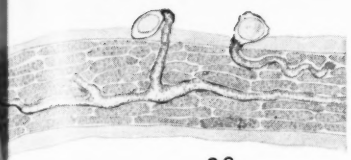

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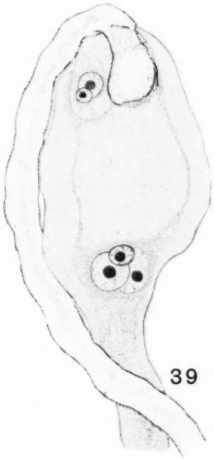

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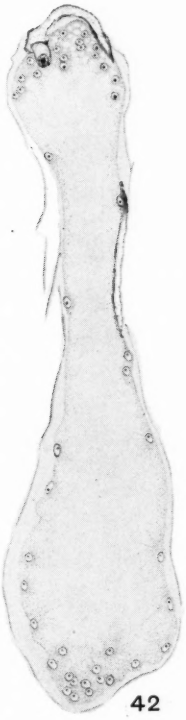

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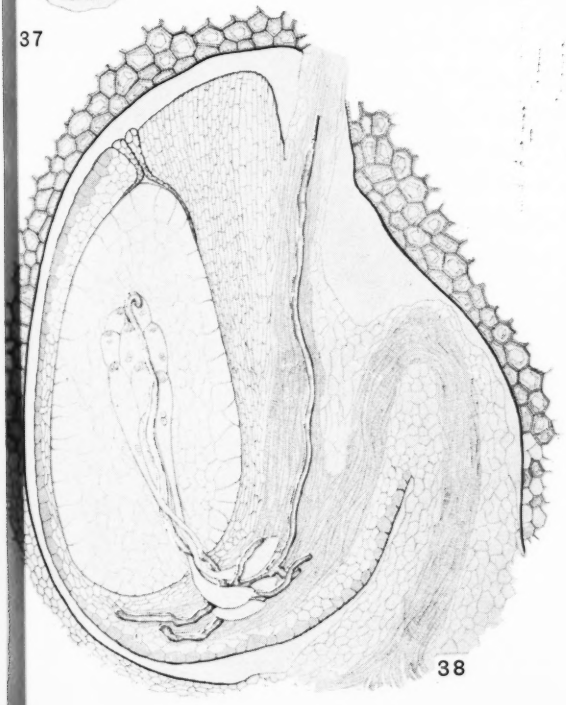

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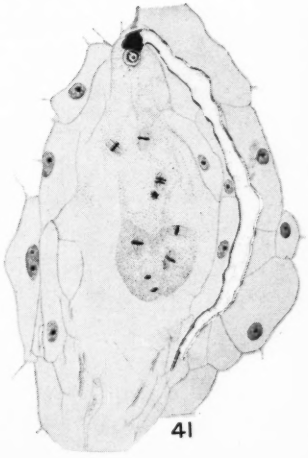

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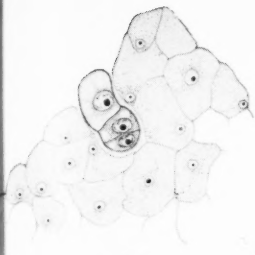

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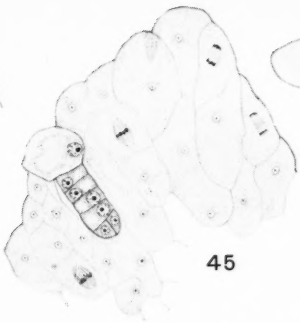

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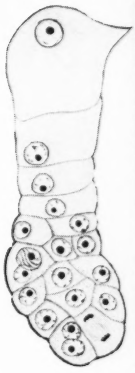

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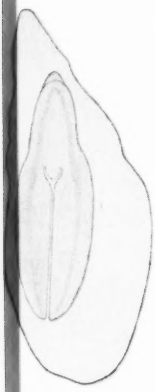

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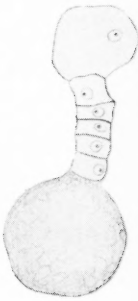

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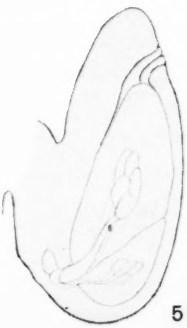

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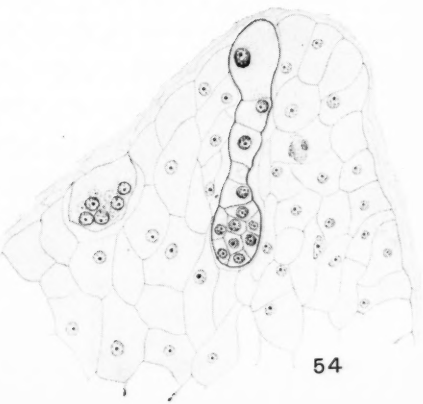

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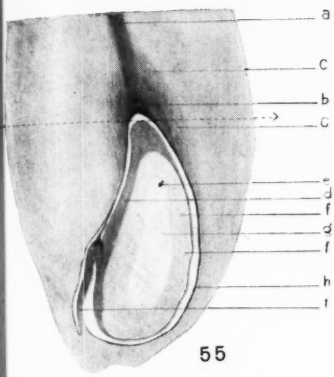

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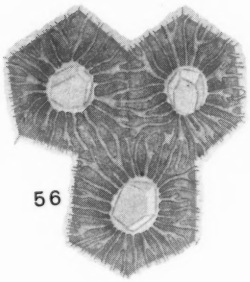
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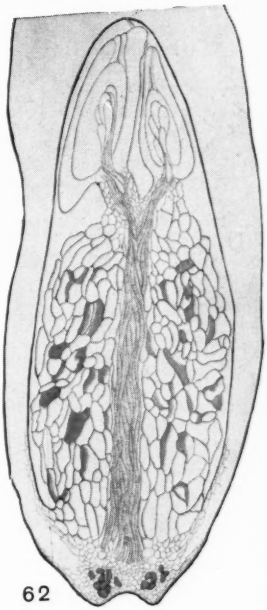
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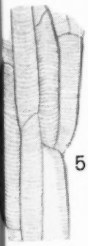
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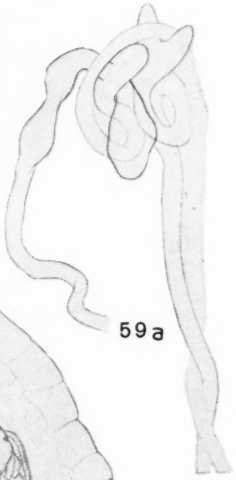
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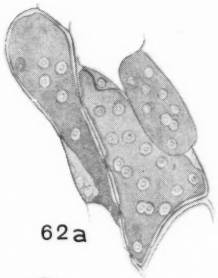
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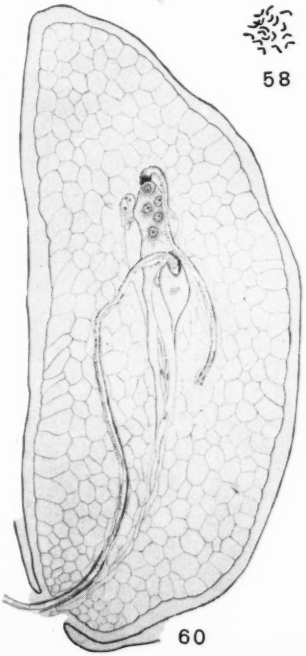
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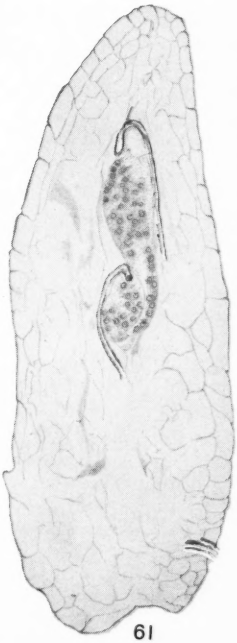
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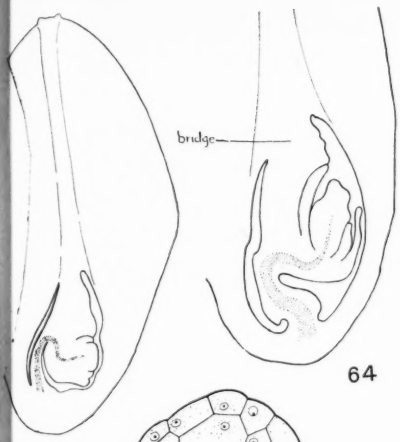

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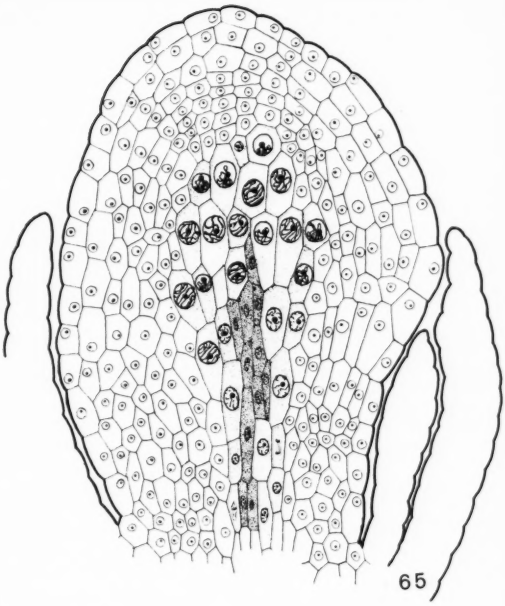

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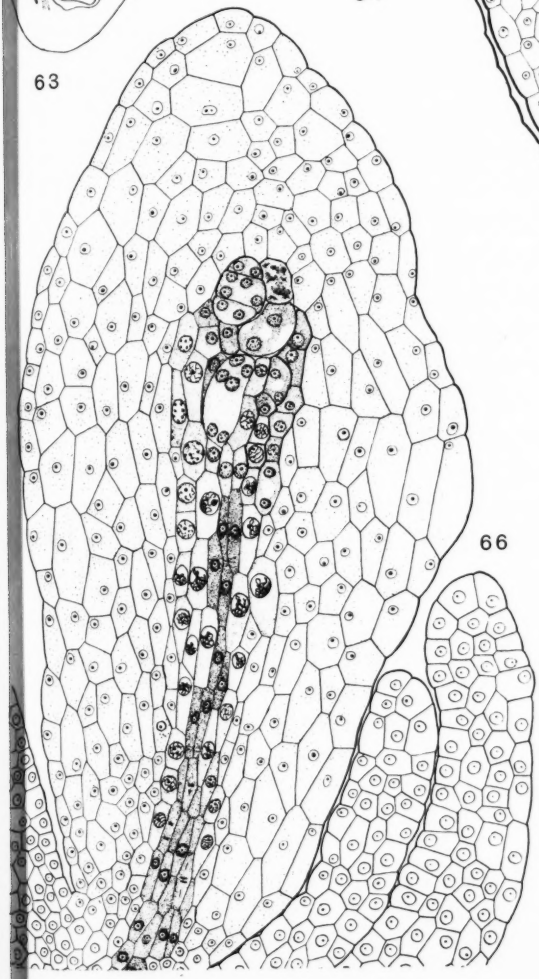

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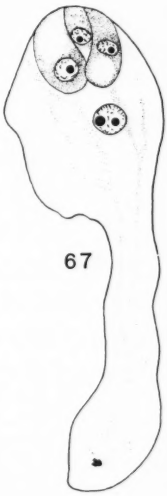

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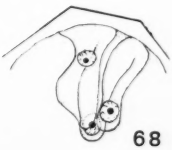

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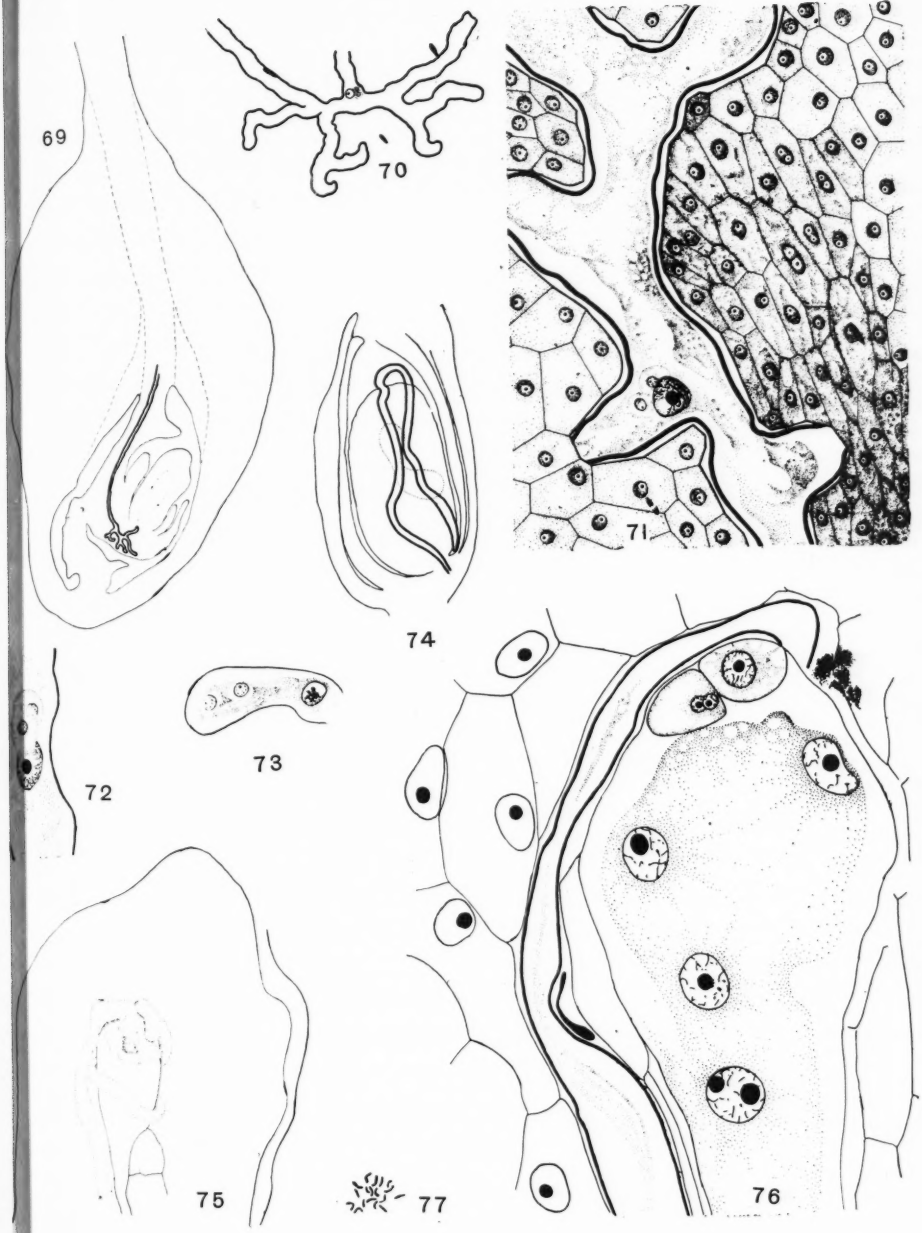
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